

CONTROLLING SAMPLE LOADING OF A SPECIMEN

BACKGROUND ART

[0001] The present invention relates to injecting a sample of a specimen.

[0002] Nowadays, modern biochemical analyzers can achieve a very high degree of
5 sensitivity and comprise a very low detection limit. Consequently the amount of
specimen used for chemical and/or biological analysis has been decreasing over the
last few years and is now in the order of some microliter. State of the art microfluidic
analyzer systems as, for example, the 2100 Bioanalyzer or the 5100 ALP Bioanalyzer
by Agilent Technologies use even smaller amounts of specimen for analysis. In these
10 analyzers a sample of the specimen is loaded and cut out for further analysis
processing. However, due to the small amount of sample loaded from the specimen,
external and sometimes not influenceable parameters like concentration gradients
within the specimen's volume, type of specimen or purification conditions might cause
a strong impact on the sample and on the analysis. This invention deals with the
15 problem of keeping the component distribution of a loaded sample similar to the
component distribution in the specimen.

DISCLOSURE OF THE INVENTION

[0003] It is an object of this invention to provide a further improved loading and
injection for a sample. The improved loading reduces the affect of concentration
20 gradients within the specimen and purification conditions.

[0004] The specimen to be analyzed normally comprises a plurality of different
components, components having different concentration within the specimen. Each of
those components might have different mass, size or even comprise an electrical
charge. A typical example of such specimen include a mixture of proteins, amino acids
25 or DNA and RNA, respectively. Particularly such protein specimen can also vary
dramatically in salt content. Additionally some of those components inherit an electrical
charge, while other are electrically neutral. For example, the specimen might comprise
a protein which is very small in size and mass but with a high electrical charge.
Another protein might comprise a higher mass and only few electrical charges.

Specimen components can also be surrounded by electrically charged carriers for analysis.

5 [0005] The mixture and huge variety of such specimen is then processed for analysis. Often only small amounts of specimen are available and microfluidic systems are used for such occurrence. In microfluidic systems the specimen is loaded into a so-called injection channel. At a specific area of the injection channel a sample is cut out (injected) from the specimen and the channel and processed for further analysis. The objective for optimal injection is that the sample composition of the injected sample exactly matches the actual composition and distribution of the specimen.

10 [0006] According to one embodiment of the invention, a sample loading device is provided. The sample loading device comprises an injection channel having a sample injection spot. The injection channel is designed for a specimen comprising fluid to be injected. Furthermore the sample loading device comprises means for transporting the specimen within the fluid along the injection channel. Thus, the specimen with its
15 different components, having a different size, mass or electrical charge is transported along the injection channel and also transported along the sample injection spot. The sample loading device further comprises a separation device for separating a sample from the specimen starting at the sample injection spot.

[0007] As mentioned before this injected sample should match the actual
20 specimen's content. If the distribution along the injection channel is mainly uniform, then the content at the sample injection point will be similar to the content of the whole specimen. However, the distribution depends on the volume used for the specimen and the way the sample is loaded into the injection channel. Therefore a control unit is provided and coupled to the loading device. The control unit is adopted for controlling
25 the loading device by analyzing a physical parameter of the fluid. It might also control the separation device. Depending on the physical parameter of the fluid the control unit regulates the loading device for loading a sample from the specimen. In other words, the time for loading the sample from the specimen is now dependent on the physical parameter of the fluid containing the specimen. This will allow the loading procedure to
30 dynamically adapt the loading process to the electrical or physical properties of the

fluid containing the specimen. The loading process is monitored by the control unit using the measurement of the physical parameter of the fluid.

[0008] In one embodiment of the invention, the physical parameter is a time-dependent electrical parameter of the fluid. Such a parameter can preferably be used if
5 the specimen within the fluid comprises electrically charged components. In an embodiment of this invention the parameter is a time-dependent resistance of the fluid along the injection channel. Measuring the resistance along the injection channel allows information about the distribution of the specimen having electrical charged components along the injection channel. In yet another embodiment of the invention
10 the parameter detected comprises a time-dependent current flow along the injection channel or a time dependent voltage drop.

[0009] In another embodiment of the invention, the injection channel comprises a specimen reservoir arranged between a first part and a second part of the injection channel. The reservoir comprises a volume for the specimen. The volume might be
15 bigger than the volume of the injection channel. A uniform distribution along the injection channel is achieved.

[00010] In yet another embodiment of the invention, the means for distributing the specimen along the injection channel are adopted for generating an electrical field along the injection channel. In an embodiment the means for distributing comprise a
20 first electrode arranged near a first end of the injection channel and a second electrode arranged near a second end of the injection channel. The sample injection spot is arranged between the first and second electrode. Applying an electrical field to the first and second electrode will pull electrically charged components of the specimen along the injection channel. In an embodiment of the invention the control unit for detecting
25 an electrical parameter of the fluid might be coupled to the first and second electrodes. The control unit might be adopted for measuring the current flowing through the injection channel, the voltage drop or the resistance, respectively, along the injection channel.

[00011] In a further embodiment of the invention, the separation device comprising a
30 separation channel is connected to the sampling injection spot. Injection channel and

- separation channel are connected together in the sample injection spot. In one embodiment of the invention they form a cross-like connection. The separation device might comprise a first electrode and a second electrode arranged in the separation channel, used for separating a sample out of the injection channel into the separation channel. Applying an electrical field of the first and second electrode of the separation channel will pull a sample from the injection spot into the separation channel thereby separating the sample from the rest of the specimen. The time for applying such an electrical field is controlled by the control unit for detecting the physical or electrical parameter of fluid.
- [00012] In another embodiment of the invention, the injection channel comprises a sieving matrix fed into the injection channel before the specimen's loading and injection, the feeding matrix comprising PDMA, acrylamide, or another polymeric substance. Such sieving matrix allows separating and distributing different components of different size and mass along the injection channel and the separation channel. The fluid comprising the sieving matrix might be used especially for electrophoresis analysis.

BRIEF DESCRIPTION OF DRAWINGS

- [00013] Other objects and many of the attendant advantages of the embodiments of the present invention will be readily appreciated and better understood by reference to the following description of preferred embodiments in connection with the accompanying drawings. It will be apparent to those skilled in the art that the drawings referenced herein are used for illustration purposes only and do not limit the scope of the invention. Features that are substantially or functionally equal or similar will be referred to with the same reference signs.
- [00014] Figure 1 shows a top view of an embodiment of the present invention.
- [00015] Figure 2 shows a different view of the same embodiment of the invention.
- [00016] Figure 3 is a concentration time diagram along the injection channel of an embodiment of the invention for different components of an injected specimen.

[00017] Figure 4 shows another embodiment of the present invention.

[00018] Figure 5 shows the separation of a specimen into its components after applying an electrical field.

[00019] Figure 6 shows a current time diagram determining the correct separation
5 time for different specimens.

[00020] Figure 7 shows the current or voltage behavior over time for a loaded specimen.

[00021] Figure 1 shows an embodiment of a microfluidic sample loading device used for electrophoresis analysis. In electrophoresis analysis an electrical field is applied to
10 a specimen comprising electrically charged components. Such components might be, for example, electrically charged amino acids or proteins or RNA or DNA or other biomolecules. Because of the electric field applied to the specimen a force moves the charged components towards the electrodes providing the electric field. Due to
15 different electrical charges of each component and different masses the moving velocity of the components will be different. Generally spoken, smaller components or components with a high electrical charge to mass ratio move faster towards the electrodes of the electrical field than larger components or components with a low charge to mass ratio. Therefore the specimen will separate into the different components.

[00022] This effect is enhanced by using a sieving matrix in which the specimen is
20 loaded, injected and separated. The sieving matrix offers additional resistance for larger components of the specimen. The moving speed is reduced in the sieving matrix depending on the size and mass of the components

[00023] Figure 5 shows an example of a standard gel electrophoresis experiment of
25 specimen having electrically charged components of different sizes and masses. A sample of the specimen is injected into a so-called separation channel S shown herein at the areas SEP. An electrical field using two electrodes (-, +) is then applied at the end of the separation channel S. As one can see at the beginning for time $T=0$, the sample is right in the middle of the separation channel S at the area SEP. Then the

electrical field is activated and the different components within the sample of the specimen become separated. After thirty seconds four different components can be distinguished. The electrical field applied to the separation channel forces the negative charged components to migrate towards the positive electrode.

5 [00024] Due to their lower mass or size, respectively, some components are moving much faster than others. After thirty seconds the electrical field is switched off. The position of the four components compared to the starting point in the middle of the separation channel gives information about the size and the mass. Component C1 was the fastest moving component followed by component C2, C3 and C4 respectively.
10 Assuming the all of the component C1 to C4 are equally charged, one might identify the component C1 as the smallest, and C4 as the biggest in size and mass. Comparing the distance of those 4 components to the starting area SEP with known samples, an identification of the size and mass or even the identification of the component is possible.

15 [00025] For automatic analysis it is easier to determine the moving time until the components C1 to C4 reach a specific point along the separation channel. The known distance from the starting area to the specific point and the measured time will then give the information needed about the size and the mass of the component. This principle of electrophoresis is used in the embodiment of the present invention
20 according to Figure 1.

[00026] The sample loading device comprises an injection channel I having a sample extraction point INJ. At the sample injection point INJ, a separation channel S is crossing the injection channel I. Both channels comprise only very small volumes in the range of micro- or even nanoliters. They may be formed in a glass or plastic body as
25 capillary vessels. The separation channel S comprises a first separation electrode Sep- in the upper part of the separation channel S and a second electrode Sep+ at the lower part of the separation channel. The electrodes are formed of a conductive material and adopted to apply a voltage or current to them. The voltage or current will generate an electrical field in the separation channel S. The lower part of the
30 separation channel S is much longer than the upper part and used to separate the

different components (anions) of the sample to be analyzed.

[00027] The injection channel I comprises a first electrode U_{inj-} on one end and a second electrode U_{inj+} on the other end. The electrodes are conductive. By applying a voltage or current to both electrodes an electrical field is generated along the injection channel I. The electrical field may be uniform and constant along the channel. This would result a constant force on the specimen. However non-homogenous electrical fields are also possible.

[00028] Between the sample injection spot INJ and the first electrode U_{inj-} a reservoir Res for the specimen is arranged. The volume of the reservoir Res is large compared to the volumes of the injection channel and the separation channel. For example the reservoir Res comprises a volume in the magnitude of a few microliter while the volumes of the injection channel I and the separation channel S comprise only a few nanoliters. In a specific example the volume of the injection channel comprises 12 nanoliters, while the volume of the reservoir Res is about 500 nanoliters. The reservoir Res comprises an inlet for feeding a specimen into the reservoir Res and an outlet VAC where vacuum for hydrodynamic specimen movement is applied.. The distance between the reservoir Res and the sample injection spot in this example is only a few hundred micrometers, for example 200 micrometers.

[00029] The injection channel as well as the separation channel is filled with a sieving matrix. The sieving matrix offers additional resistance for the components of the specimen, when moving towards the electrodes after applying an electrical field. The sieving matrix might comprise PDMA (poly-N,N-dimethyl-acrylamide), polyacrylamide, polyethylene oxide, polyvinylpyrrolidone, hydroxypropylmethylcellulose or other polymeric matrices.

[00030] The specimen is then drawn into the reservoir hydrodynamically by applying a vacuum on the outlet VAC, moving the specimen into the reservoir. Once the reservoir and the inlet and outlet areas are filled by the specimen the vacuum is turned off and an electrical field is applied across the first electrode U_{inj-} and the second electrode U_{inj+} of the injection channel I.

[00031] The different components within the specimen will now electrokinetically move along the injection channel towards the first electrode or the second electrode. For example, the negative charged components of the specimen will be moving towards the second electrode U_{inj+} . At a specific point in time the portion of the specimen at the sample injection spot INJ is injected into the separation channel S. This is done by modifying the electrical field of the first and second electrode U_{inj-} and U_{inj+} of the injection channel I and applying a second electric field using the separation electrodes $Sep-$ and $Sep+$. Thereby a sample of the specimen at the injection spot INJ is cut out and injected into the separation channel S. The components within that sample will now separate due to different velocities while moving along the separation channel S towards the separation electrode $Sep+$ and the measurement point M.

[00032] Another view of the sample loading device according to Figure 1 can be seen in Figure 2. The separation channel SEP is perpendicular to the drawing plane only showing the sample injection point INJ. One can also see the injection electrode U_{inj-} and U_{inj+} of the injection channel I. They are placed in an inlet area, which is connected to the injection channel I. Those areas are adopted to be filled by the sieving matrix. The specimen is drawn into the reservoir by an inlet area on the lower right of the reservoir Res.

[00033] When applying an electric field along the injection channel I the resulting driving force is acting on all charged components within the specimen. Those components will be moving through the sieving matrix within the injection channel with different velocities, depending on their size and charge-mass ratio. Smaller components or components with a high charge-mass ratio will move faster along the injection channel I than larger components or components with a lower charge-mass ratio. The distribution of different components in the specimen over time at the sample injection spot can be seen in Figure 3.

[00034] In this example the specimen comprises Cl^- (chloride), which is normally incorporated in buffers or other sample components. Furthermore the specimen comprises two different proteins P1 and P2 that are engulfed within a negatively

charged carrier molecule like sodium-dodecyl sulfate (SDS). The protein component P2 is larger than the first protein P1. The curves show the distribution or concentration function over time on the sample injection spot INJ when an electrical field across the two electrodes Uinj- and Uinj+ of the injection channel I is applied. Since the sodium and chloride ions are very small they are moving very fast along the injection channel. The maximum concentration of Cl-anions on the sample injection spot occurs at the time T1. However, the concentration ratio of the first protein P1 and the second protein P2 at this time T1 is different from the normal concentration ratio in the specimen. While the concentration for the first component P1 is roughly one half of the maximum concentration, the concentration of the second protein P2 is less than a quarter of the maximum concentration. The concentration ratio of P1 and P2 at this time are therefore different compared to the overall concentration ratio of the specimen.

[00035] In other words, the parameters for loading a sample to the sample injection spot have to be chosen very carefully in order to achieve in the sample (to be analyzed) the same concentration ratio for protein P1 and P2 as in the original specimen.

[00036] At the loading time t(LS) the concentration of protein P1 and protein P2 have already reached their maximum (plateau) and do not change anymore. The concentration ratio at this time now represents the concentration ratio in the original specimen. Therefore the time t(L) is considered to be the optimum for loading the sample on the sample injection spot into the separation channel for further analysis.

[00037] To determine the correct loading time t(LS) for the specimen to be analyzed the current across the injection channel I or the resistance across the injection channel I are measured over time. Upon application of the electrical field to move the specimen's anions across the injection channel towards the Uinj+ electrode the channel resistance decreases while the current flow along the injection channel I increases. This behavior can be seen in frame B to D of figure 7. The voltage along the injection channel, showing in frame B, decreases from roughly 1300 Volts to about 1000 V. At the same time the current along the injection channel is increasing, as shown in frame C. The reasons for this behavior are that the specimen ions are moving

from the reservoir into the injection channel, which is so far only filled with sieving matrix and the ions therein. The current transporting ion density increases along the injection channel. The total number of ions between the two electrodes U_{inj-} and U_{inj+} along the injection channel is thus increasing.

5 [00038] At a certain point in time the fast moving anions, for example the chloride anions of the specimen, have reached the second electrode U_{inj+} . At this point the density of ions along the injection channel I has reach a maximum and is now mostly homogenous along the injection channel. The channel resistance in Frame D is now at a minimum point. After that the channel resistance in frame D of Figure 6 starts to rise
10 again. The longer the injection voltage is applied the more ions are drawn to the electrodes U_{inj-} and U_{inj+} . The ion density in the injection channel decreases again with the sample reservoir being depleted.

[00039] These observations lead to a loading time depending on the current or the resistance along the injection channel I. If the loading time is too short, the specimen
15 components of high molecular weight may be underrepresented. Vice versa if sample loading is allowed to proceed too long the low molecular weight components are underrepresented, because the majority already reached the second injection electrode U_{inj+} . However, in this example, the minimum in overall channel resistance indicates a good distribution of all ion types within the injection channel I.

20 [00040] Therefore, the channel resistance or the current during the injection procedure is measured. If a maximum in the current or a minimum in the resistance is reached sample loading is stopped by changing the electrical field generated by the two electrodes U_{inj-} and U_{inj+} . Then the separation electrodes $Sep-$ and $Sep+$ are switched on thereby injecting a sample of the specimen. Figure 6 shows a current time
25 measurement for different specimens comprising two different ion concentrations.

[00041] The measurements show the current along the injection channel I versus time. The specimen used for the measurement in frame A comprises a very low NaCl concentration compared to a specimen used in frame B, which include a high concentration of NaCl. After starting the loading procedure by switching on the two
30 injection electrodes, the loading time is measured. One can see that the loading time

of a specimen with low concentration of NaCl is approximately 3.3 seconds, while the loading time of the high NaCl concentration specimen in frame B is approximately 11.5 seconds. Furthermore the current increase as well as the maximum value is also different. It is apparent that increasing the concentration of NaCl will also increase the maximum current flowing along the injection channel as well as a stronger increase during the loading time.

[00042] Figure 4 shows an embodiment of a sample loading device according to an embodiment of the invention. The sample loading device comprises an injection channel I, a sample injection spot INJ in the injection channel I. A separation channel S is connected to the sample injection spot INJ. The injection channel I as well as the separation channel S is filled with a sieving gel matrix comprising PDMA or acrylamide or any other polymer. The gel matrix comprises a relatively high viscosity and acts as a flow resistance for the specimen components depending on the specimen components' size and mass. The matrix comprises a high specific resistance, which can be significantly reduced by injecting ions into the sieving matrix. On the ends of the injection channel I and the separation channel S conductive electrodes are placed into the channel. The two electrodes of the injection channel I are connected to a voltage generator UIN and to a control unit RD. The control unit RD measures the resistance across the injection channel I. It also controls the voltage generator UIN. The two electrodes of the separation channel S are connected to a second voltage generator SG, which is also controlled by the control unit RD. Consequently the control unit RD is connected to the voltage generator UIN and the second generator SG. After injecting a specimen into the injection channel the control unit RD selects the voltage generator UIN, which applies a voltage of roughly 2500 Volt across the injection channel I. Particularly a potential of +300 Volts supplied at the first electrode I1 of the injection channel I and a second potential of 2200 Volts is applied to the second electrode I2.

[00043] The cations are now moving towards the first electrode I1 while the anions are drawn towards the second electrode I2. At the same time the current through the injection channel I increases while the resistance measured by the control unit RD starts to decrease. At a certain point in time the resistance across the injection channel reaches its minimum and starts to increase again. The control unit RD sends a signal

to the voltage UIN to switch off the injection voltage. The different components loaded into the injection channel are now distributed almost homogeneously at the injection spot whereas their distribution other parts of may significantly vary. Specifically the distribution of different components within the specimen at the sample injection spot is
5 considered homogeneous and having a concentration ratio similar to the original content of the specimen.

[00044] The control unit RD triggers the separator generator SG to switch on the separation electrodes. A separation voltage is applied to the separation electrodes thereby driving the anions and cations of the sample at the sample injection spot into
10 the separation channel S.

The sample loading device as well as the sample loading method is not limited to electrophoresis analysis described as an example herein. The loaded sample can also be processed by different means of analysis without limiting the scope of protection. For example, if channel geometries differ from the system described here (especially
15 distances between Res & INJ), the optimum time point for sample injection may no longer be characterized by the lowest channel resistance but by another point on the channel resistance/conductivity curves. The best suited injection time character can be empirically determined, and the control units may then be programmed appropriately. The sample movement inside the capillaries may very well be controlled by
20 pressure/vacuum instead of electrically. Still, electric information like channel resistances, currents, etc. may still be used to determine the optimal time point for sample injection.